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Editorial
Articular cartilage nuclear receptors: an emerging target for treatment of osteoarthritis

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A major focus of translational research in osteoarthritis (OA) is discovery of the factors that regulate articular cartilage homeostasis. The hope of many investigators, and patients alike, is that new insights into the regulation of cartilage metabolism will lead to the development of therapeutic agents that will slow or halt the progression of cartilage destruction in OA. There is substantial evidence that an imbalance in anabolic and catabolic activity in cartilage, favoring catabolism of the extracellular matrix, plays a central role in the loss of cartilage that is characteristic of OA[1,2]. It might be debated whether targeting cartilage metabolism will affect progression of a disease that involves altered biomechanics and affects multiple joint tissues in addition to cartilage. However, until we have clinical trial evidence to suggest otherwise, articular cartilage metabolism will continue to be a major therapeutic target.

The articular chondrocytes, which are responsible for both synthesis and degradation of the cartilage matrix, are regulated by a host of signals generated by growth factors, cytokines/chemokines, changes in the extracellular matrix, and mechanical stimulation[1,2]. A multitude of signaling pathways, activated by these diverse stimuli, act in concert to regulate chondrocyte expression and synthesis of matrix proteins as well as enzymes that degrade matrix proteins. The signaling pathways that regulate chondrocyte matrix synthesis and degradation represent potential therapeutic targets. Limited pre-clinical studies in animal models have provided proof of concept for a positive effect on articular cartilage with inhibition of certain cell signaling proteins, such as the mitogen-activated protein kinases, that are central to multiple pathways[3]. However, specificity of small molecule inhibitors of cell signaling proteins and toxicity related to off target effects have been a concern.

Another approach to modulating intracellular signaling pathways that regulate gene expression is to target nuclear receptors. Nuclear receptors bind a large number of ligands to mediate a diverse array of cellular responses generated by hormonal and metabolic signals. Nuclear receptors act as transcriptional regulators (activators or repressors) and contain certain conserved structural domains that mediate ligand binding, binding to DNA, and binding to regulatory co-factors including other nuclear receptors and transcription factors[4]. There are 48 known human nuclear receptors that function as homo-and/or heterodimers or less commonly as monomers[5].

Nuclear receptors bind to a diverse group of ligands that include lipids such as fatty acids (peroxisome proliferator-activated receptors, PPARs) and cholesterol metabolites (liver X receptor, LXR), fat soluble vitamins (vitamin D receptor, VDR; retinoic acid receptor, RAR), hormones such as estrogen (estrogen receptors, ER), steroids (glucocorticoid receptor, GR) and thyroid hormone (thyroid hormone receptor, TR), and xenobiotics (pregnane X receptor, PXR also called steroid and xenobiotic sensor, SXR). Because the retinoid X receptor (RXR) forms heterodimers with several different nuclear receptors and regulates multiple signaling pathways it is often called a ''master regulator''. Approximately half the members of the nuclear receptor family do not have a known ligand and are often termed ''orphan receptors'' (for example Nur-related factor 1, NURR1)[4].

Nuclear receptors are considered by the pharmaceutical industry to be good drug targets. Ligands for the nuclear receptors in the endocrine subfamily, such as thyroid hormone, various steroid hormones, vitamins A and D, and estrogen, are widely used for a number of conditions and some are potential therapies for OA. Due to evidence suggesting increased progression of knee OA in people with low serum levels of vitamin D[6] and the well documented effects of vitamin D on bone, there are two ongoing clinical trials (listed on clinicaltrials.gov) examining the ability of vitamin D to slow progression of knee OA. There is evidence, although not conclusive, that postmenopausal estrogen loss may contribute to the progression of OA in women and, at least in animal models, estrogen replacement therapy initiated at the time of surgically induced menopause can slow disease progression[7,8].

The PPARs have received much attention as therapeutic targets. Successful drugs to date include the thiazolidinediones for type II diabetes that activate PPARγ and serve as insulin-sensitizers and the fibrates that activate PPARα to lower serum lipids. Given their important functions as integrators of metabolic and inflammatory signaling pathways[9], PPARs as well as LXRs could represent therapeutic targets in OA. *In vitro* studies have suggested that agonists of

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PPARγ have an anti-catabolic effect in articular cartilage, supporting their potential use as disease-modifying drugs for OA[10,11]. Indeed, animal model studies have demonstrated that the PPARγ agonist pioglitazone can reduce progression of surgically induced OA in guinea pigs[12] and dogs[13] but trials in humans are not yet available.

Looking for additional nuclear receptor targets in OA, Collins-Racie and colleagues report, in this issue of *Osteoarthritis and Cartilage*, an analysis of the expression of all known nuclear receptors in both normal and osteoarthritic human articular cartilage[14]. Out of the 48 known nuclear receptors, 31 were surprisingly found to be expressed at detectable levels in cartilage. Expression of 23 nuclear receptors was significantly different between RNA samples isolated from normal and OA cartilage. Most of the nuclear receptors identified in cartilage were downregulated in OA tissue relative to normal tissue with the exception of RARβ and RARγ which were significantly upregulated. Given the potential for increased RAR activity to down-regulate cartilage matrix gene expression[15], the finding of increased RAR in OA cartilage might be functionally significant.

An interesting technical finding from the analysis was that all but four of the nuclear receptors (Rev-Erbα, LXRβ, RXRα, and GR) were expressed at levels below the detection limits of standard microarray analysis using Affymetrix GeneChips. The remaining 27 were detected using a more sensitive custom TaqMan® real-time polymerase chain reaction (PCR) array. This finding supports the concept that important genes can be missed when relying only on standard microarrays for initial screening of gene expression. However, the significance of detecting RNA coding for the 31 nuclear receptors in chondrocytes using a sensitive real-time PCR expression analysis will not be known until further experiments are performed. It will be important to determine which of these nuclear receptors are present at the protein level and which are functional in chondrocytes.

There is data, albeit limited, in the prior literature and in the new results presented by Collins-Racie *et al.* to suggest that at least some of the nuclear receptors identified in this study are functional in cartilage. Besides the previous work noted above on PPARγ and ERα and ERβ, the orphan nuclear receptor NURR1 has been previously identified in human articular cartilage where overexpression of NURR1 by transient transfection was found to repress expression of matrix metalloproteinase (MMP)-1, -3, and -9[16]. Contrary to the results of Collins-Racie *et al.* which found no significant difference in NURR1 expression between normal and OA, the previous study by Mix *et al.* noted a significant increase in OA tissue, although this result appeared to be driven by a few high expressors. Others have provided evidence in chondrocytes for functional VDR and RXR[17,18], Rev-ErbAα[19], RAR[15] and in growth plate chondrocytes RAR-related orphan receptor (ROR)α[20].

Collins-Racie *et al.* chose to focus additional experiments on LXRα and LXRβ which were significantly decreased in OA relative to normal cartilage. Decreased expression of three known LXR target genes was also noted in OA cartilage adding support to functional significance. Importantly, addition of a synthetic LXR agonist (TO901317) stimulated expression of the LXR target genes in OA cells and inhibited proteoglycan release (as a measure of proteoglycan degradation) from OA cartilage explants as well as explants treated with the cytokines interleukin-1 and oncostatin M. The LXR agonist also caused expression of LXR itself to increase in a positive feedback manner suggesting that the use of such an agonist could restore normal levels of LXR in OA cells and potentially inhibit proteoglycan degradation.

The imbalance in anabolic and catabolic activities in cartilage that is characteristic of OA appears to be driven by an increase in levels of numerous inflammatory mediators including cytokines, chemokines, prostaglandins, leukotrienes, and reactive oxygen species, among others[1,2]. Targeting these factors separately will be difficult and attempts to do so have not met with much success. Given the knowledge that activation of several of the nuclear receptor family members including PPARγ, LXR, RXR, ER, and NURR1 can have anti-inflammatory and/or anti-catabolic effects, and given the relative safety profile of nuclear receptor agonists studied to date, we may expect an emergence of new therapies targeting nuclear receptors in OA. Clearly, further work is indicated to determine the nuclear receptors that would be the best targets in OA but the study by Collins-Racie *et al.* sheds light on where further efforts may lead.

Conflict of interest

None declared.

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